

¹ Abstract

- ² 1. For many vertebrates, urban environments are characterised by frequent environmental stressors. Coping with such stressors can demand that urban individuals activate energetically costly physiological pathways more regularly than rural-living conspecifics. However, urban environments also commonly demand appreciable expenditure toward thermoregulation, owing to their often extreme climatic variation. To date, whether and how vertebrates can balance expenditure toward both the physiological stress response and thermoregulation, and thus persist in an urbanising world, remains an unanswered and urgent question among ecologists.
- ¹⁰ 2. We tested whether changes in body surface temperature (T_s) and peripheral heat loss (q_{Tot}) that accompany the stress response: 1) endow urban individuals with an enhanced capacity to conserve heat in the cold, and dissipate heat in the warmth relative to rural conspecifics, and 2) meet essential criteria for evolutionary responses to selection (here, variability among, and consistency within individuals).
- ¹⁵ 3. Using the black-capped chickadee ($n = 19$) as a model species, we show that neither rapid nor chronic changes in T_s and q_{Tot} following stress exposure differed between urban- and rural-origin individuals ($n_{urban} = 9$; $n_{rural} = 10$). Nevertheless, we do find that stress-induced changes in T_s and q_{Tot} are highly repeatable across chronic time periods ($R_{T_s} = 0.61$; $R_{q_{Tot}} = 0.67$) and display signatures of stabilising or directional selection (i.e. reduced variability and increase repeatability relative to controls).
- ²¹ 4. Our findings suggest that, whilst urban individuals appear no more able to balance expenditure toward thermoregulation and the stress responses than rural conspecifics, the capacity to do so may still be subject to selection in chickadees.

²⁴ **Keywords;** Flexibility, Stress, Thermoregulation, Repeatability

²⁵ 1 | INTRODUCTION

²⁶ Over the past 70 years, the global human population has increased by approximately
²⁷ 350% (or approximately 5.1 billion; United Nations, Department of Economic and
²⁸ Social Affairs, Population Division 2019). Unlike in previous centuries, the major-
²⁹ ity of individuals (nearly 54%) now reside in urban environments, and global trends
³⁰ strongly suggest that urban living will increasingly become the norm (reviewed in
³¹ Lerch 2017). Consequently, land area designated for urban utility is expanding at
³² unprecedented rates and will probably continue to do so over the coming decades
³³ (Angel *et al.*, 2011). Such expansion cannot, however, occur in a vacuum, and has
³⁴ thus contributed to the widespread reduction in habitat availability and quality for
³⁵ many species (Grimm *et al.* 2008; Seto *et al.* 2012; Freeman *et al.* 2019; lay literature:
³⁶ Thomas 2017). For this reason, understanding whether these species can adapt and
³⁷ persist within modern city-scapes has become a growing priority among modern
³⁸ ecologists and conservationists (e.g. Birnie-Gauvin *et al.* 2016; Ouyang *et al.* 2018).

³⁹ Yet habitat loss or degradation are not the only challenges faced by species in ur-
⁴⁰ ban environments. Indeed, urban environments regularly present acute challenges,
⁴¹ including noise, frequent human interaction, vehicle traffic, and in some cases, el-
⁴² evated depredation and inter- and intra-specific competition (Johnson *et al.* 2012;
⁴³ Hernández-Brito *et al.* 2014; Newsome *et al.* 2015; Vincze *et al.* 2017; reviewed in
⁴⁴ Lowry *et al.* 2013). Coping with these acute challenges can demand that individ-
⁴⁵ uals within urban environments activate self-preserving physiological responses (i.e.
⁴⁶ fight-or-flight responses) more regularly than rural-living conspecifics (Bonier 2012;
⁴⁷ Watson *et al.* 2017; albeit, often with reduced intensity; Partecke *et al.* 2006; French
⁴⁸ *et al.* 2008; but see Fokidis *et al.* 2009). Whilst such demands need not inherently trans-

⁴⁹ late to a loss of fitness among urban individuals, laboratory studies suggest that their
⁵⁰ daily metabolic costs are probably raised owing to increased allostatic load (Depke
⁵¹ *et al.*, 2008; Jimeno *et al.*, 2017). In turn, these elevated metabolic demands may en-
⁵² hance susceptibility to wear and tear when resources are restricted or are required to
⁵³ be allocated elsewhere (Romero *et al.*, 2009; Breuner & Berk, 2019).

⁵⁴ Beyond urban development, many of today's species face additional and indirect threats
⁵⁵ associated with a growing human population. Effects of anthropogenic climate change
⁵⁶ on species distribution and trait expression, for example, have now been argued for
⁵⁷ nearly all taxa (e.g. Barton *et al.* 2016; Mainwaring *et al.* 2017; Pacifici *et al.* 2017;
⁵⁸ Wan *et al.* 2018), and concerns over the ability of species to adjust to rising and in-
⁵⁹ creasingly variable ambient temperatures (Vasseur *et al.*, 2014) have been well articu-
⁶⁰ lated (e.g. Rutschmann *et al.* 2015; Radchuk *et al.* 2019). In endotherms, increases in
⁶¹ both maximal ambient temperature and variability of ambient temperatures can bear
⁶² notable thermoregulatory costs (Pendlebury *et al.*, 2004; du Plessis *et al.*, 2012; Smit
⁶³ *et al.*, 2018), with those associated with the former being particularly severe in urban
⁶⁴ environments (Arnfield, 2003). These costs, coupled with expected increases in sus-
⁶⁵ ceptibility of wear and tear, beg important questions of whether and how endotherms
⁶⁶ may cope with increasingly urbanised environments in the face of a rapidly changing
⁶⁷ climate (discussed in Pautasso 2012; Argüeso *et al.* 2015; Brans *et al.* 2017).

⁶⁸ To date, several empirical studies have shown that endotherms may adjust their super-
⁶⁹ ficial blood-flow, and thus, their body surface temperatures (henceforth, " T_s ") when
⁷⁰ exposed to stressors (e.g. Blair *et al.* 1959; Yokoi 1966; Nord & Folkow 2019; Winder
⁷¹ *et al.* 2020). In some species, these changes in T_s appear to endow individuals with
⁷² greater heat conservation in the cold, and greater heat dissipation in the warmth, thus

73 reducing their demands for costly thermogenesis or evaporative cooling respectively
74 (Jerem *et al.* 2018; Robertson *et al.* 2020a; Winder *et al.* 2020). In this way, total en-
75 ergetic expenditure may be balanced in challenging environments by allocating en-
76 ergy toward more immediate and higher-cost threats (e.g. the perceived stressors)
77 and away from less immediate and lower-cost threats (e.g. thermal challenges; Jerem
78 *et al.* 2018; Robertson *et al.* 2020a). In urban environments, where individuals reg-
79 ularly contend with both physical and thermal challenges, such flexibility of T_s and
80 peripheral heat loss (here, non-evaporative heat-loss; henceforth, " q_{Tot} ") could be par-
81 ticularly advantageous, with those capable of enhanced flexibility (particularly during
82 stress exposures) being better able to balance energy expenditure and, therefore, be-
83 ing favoured by selection (Parsons, 2005). Nevertheless, the potential for selection to
84 act on flexibility of T_s and q_{Tot} in response to stressors requires that these traits are
85 both variable among individuals, and consistent within individuals (i.e. "repeatable";
86 reviewed in Boake 1989; Wolak *et al.* 2012). Over the past two decades, numerous
87 studies have reported moderate to high degrees of repeatability among traits associ-
88 ated with the stress response and whole-animal metabolism (Nespolo & Franco 2007;
89 Rensel & Schoech 2011; Müller *et al.* 2018; Boratyński *et al.* 2019; but see Ouyang
90 *et al.* 2011). Whilst these finding strongly suggest that stress-induced changes in T_s
91 and q_{Tot} are also likely to be repeatable in endotherms, the degree of this repeatability
92 remains largely unclear (but see Careau *et al.* 2012).

93 Using the black-capped chickadee (*Poecile atricapillus*; henceforth "chickadees") as a
94 model species, we tested whether flexibility of both T_s and q_{Tot} during stress exposure:
95 1) meet critical first criteria for responsiveness to selection, and 2) offer opportunities
96 for endotherms to cope with the increased allostatic and thermoregulatory costs of
97 an urbanising environment. More specifically, we hypothesised that stress-induced

98 changes in both T_s and q_{Tot} : 1) are variable among, and consistent within individu-
99 als, 2) provide evidence of current or past selection, and 3) differ between individuals
100 captured from urban and rural environments. Accordingly, we first predicted that
101 stress-induced changes in both T_s and q_{Tot} would be repeatable among individuals.
102 Because thermal responses to stress exposure can be acute (e.g. minutes to hours:
103 Jerem *et al.* 2015; Andreasson *et al.* 2020; Winder *et al.* 2020) or chronic (e.g. days: de
104 Aguiar Bittencourt *et al.* 2015; Herborn *et al.* 2018), and responses across each time-
105 period may provide energetic benefits by enhancing heat dissipation or relaxing costs
106 of thermogenesis (Jerem *et al.* 2018; Herborn *et al.* 2018; Winder *et al.* 2020; Robert-
107 son *et al.* 2020a), we predicted that both acute and chronic changes in T_s and q_{Tot} ac-
108 companying the stress response would be repeatable among individuals in our sample
109 population. Next, because traits subject to previous or current selection (here, direc-
110 tional or stabilising) are thought to display lower variability and higher repeatability
111 than those that are selectively neutral (e.g. Gibson & Bradley 1974; Lande & Arnold
112 1983; Boake 1989; Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001), we predicted
113 that both T_s and q_{Tot} of chickadees would be less variable and more repeatable dur-
114 ing stress exposure treatments than during control treatments, after controlling for
115 predictable environmental effects on heat loss (e.g. ambient temperature and relative
116 solar radiation). Finally, because the combined energetic costs of stress exposure and
117 thermoregulation are expected to be higher in urban environments when compared
118 with rural environments (i.e. in the absence of phenotypic differences between urban
119 and rural individuals; discussed above), we predicted that the magnitude of both acute
120 and chronic changes in T_s and q_{Tot} that accompany stress exposures would be larger
121 among urban-origin individuals than rural-origin individuals.

122 To test our predictions, we exposed chickadees captured from urban and rural envi-

123 environments to both repeated stressors and control conditions across an ambient temper-
124 ature gradient whilst monitoring rapid and long-term changes in T_s and q_{Tot} by infra-
125 red thermography. In small birds, surface tissues at the periorbital region (henceforth
126 "eye region") are thought to play a critical role in environmental heat exchange (e.g.
127 Hill *et al.* 1980; Powers *et al.* 2015) and both temperature of, and heat loss from this re-
128 gion have previously been shown to respond to stress exposure (e.g. Jerem *et al.* 2015;
129 Ikkatai & Watanabe 2015; Herborn *et al.* 2018; Robertson *et al.* 2020a). We, therefore,
130 chose to use temperature of, and heat loss from, the eye region as our indicators of T_s
131 and q_{Tot} in this study.

132 The capacity of vertebrates to cope with the combined pressures of urbanisation and
133 anthropogenic climate change has been questioned many times (Pautasso, 2012; Argüeso
134 *et al.*, 2015; Brans *et al.*, 2017). The proximate physiological mechanisms by which
135 vertebrates (here, endotherms) may do so, however, are seldom explored. Ours study,
136 therefore, represents a critical step forward in how ecologists might test the capacity
137 of vertebrates to adapt an increasingly human-modified world.

138 2 | MATERIALS AND METHODS

139 All methods used for animal capture, sampling, and experimental treatment were ap-
140 proved by the Trent University Animal Care Committee (AUP # 24614) and Envi-
141 ronment and Climate Change Canada (permit # 10756E).

¹⁴² 2.1 | Capture, transport, and housing of experimental animals

¹⁴³ Chickadees ($n = 20$; $n = 10$ females, $n = 10$ males) used for this experiment were
¹⁴⁴ captured within a 100 km^2 region of south-western Ontario, Canada, between the
¹⁴⁵ months of March and April in 2018. To minimise the possibility of kinship be-
¹⁴⁶ tween individuals within our sample population, capture efforts were divided across
¹⁴⁷ six distinct locations (three urban and three rural), each separated by a minimum
¹⁴⁸ distance of 15 km. Urban capture locations included the downtown regions of the
¹⁴⁹ cities of Brantford (43.1345°N , 80.3439°W), Cambridge (43.3789°N , 80.3525°W),
¹⁵⁰ and Guelph (43.3300°N , 80.1500°W), whilst rural capture locations included the town-
¹⁵¹ ships of Corwhin (43.5090°N , 80.0899°W), Erin (43.7617°N , 80.1529°W), and Cayuga
¹⁵² (42.9797°N , 79.8745°W) (SFigure 1). A difference in the mean degree of urbanisation
¹⁵³ between urban and rural capture locations was validated using methods similar to
¹⁵⁴ Thompson et al (2018; see Appendix; SFigures 2–4).

¹⁵⁵ All individuals were captured using modified potter traps (dimensions [$\text{L} \times \text{W} \times \text{H}$]
¹⁵⁶ = $90 \times 70 \times 70 \text{ cm}$), baited with sunflower seeds and suet on the day of capture.
¹⁵⁷ To further draw individuals to trap locations, we alternately broad-casted chickadee
¹⁵⁸ breeding songs and alarm calls from a remote call-box (FoxPro™ Patriot; Lewisville,
¹⁵⁹ PA, USA) until at least one individual approached a potter trap by ≤ 4 meters. Upon
¹⁶⁰ capture, chickadees were blood sampled (approximately $50 \mu\text{L}$) by brachial venipunc-
¹⁶¹ ture and capillary tube collection, then fitted with one stainless steel, numbered leg
¹⁶² ring (size 0) and a unique combination of two, coloured, Darvic leg rings (size 0) for
¹⁶³ future identification. Each individual was then measured (mass to the nearest 0.1 g
¹⁶⁴ using an electronic scale, and, wing cord to the nearest 0.1 mm, left outer tarsus to
¹⁶⁵ the nearest 0.1 mm, and head-to-bill to the nearest 0.1 mm using analogue calipers)

¹⁶⁶ and secured in a covered flight enclosure (dimensions $[L \times W \times H] = 30 \times 30 \times$
¹⁶⁷ 15 cm) for transportation to our long-term housing facility (Ruthven Park National
¹⁶⁸ Historic Site, Cayuga, Ontario; ≤ 90 km drive). Blood samples were preserved in a
¹⁶⁹ small volume of Queen's Lysis buffer ($500 \mu\text{L}$; Seutin *et al.* 1991) for use in genetic
¹⁷⁰ sexing (described in Robertson *et al.* 2020a) and were held on ice until storage at 4°C
¹⁷¹ was possible (≤ 2 hours).

¹⁷² Upon arrival to our long-term housing facility, chickadees were haphazardly dis-
¹⁷³ tributed among four, visually isolated flight enclosures ($n = 5$ per enclosure; dimen-
¹⁷⁴ sions $[L \times W \times H] = 1.83 \times 1.22 \times 2.44$ m), each equipped with one white cedar tree
¹⁷⁵ (*Thuja occidentalis*), two perching branches (raised to approximately 1.50 and 1.80 m
¹⁷⁶ above ground) and a raised feeding platform (400 cm^2) at which food was provided
¹⁷⁷ *ad libitum* through an opaque hinged door for the duration of the experiment (Fig-
¹⁷⁸ ure 1). Food provided included sunflower seed, safflower seed, shelled peanuts, boiled
¹⁷⁹ egg, apple pieces, house crickets (*Acheta domesticus*), meal worms (*Tenebrio molitor*)
¹⁸⁰ and Mazuri (St Louis, MO, USA) Small Bird Maintenance diet. Water was also pro-
¹⁸¹ vided *ad libitum* across our experiment through opaque hinged doors. All individuals
¹⁸² were given a minimum of 2 weeks to acclimate to enclosures and social groups prior
¹⁸³ to onset of experimentation.

¹⁸⁴ 2.2 | Experimental stress exposure

¹⁸⁵ To test repeatability of stress-induced thermal responses within and among individuals,
¹⁸⁶ we used a paired experimental design wherein each individual was exposed to both a
¹⁸⁷ thirty-day control treatment and a thirty-day stress exposure treatment, with treat-
¹⁸⁸ ments separated by an additional two-day control period (total experimental duration

¹⁸⁹ = 62 days). To control for possible effects of treatment order on stress-induced thermal
¹⁹⁰ responses, half of our sample population ($n = 10$ across two flight enclosures) was ex-
¹⁹¹ posed to control treatments followed by stress-exposure treatments, whilst the second
¹⁹² half of our sample population ($n = 10$ across two flight enclosures) was concurrently
¹⁹³ exposed to a reversed treatment order (i.e. stress-exposure treatments followed by
¹⁹⁴ control treatments).

¹⁹⁵ Each day, individuals within stress exposure treatments were exposed to 5 or 6 exper-
¹⁹⁶ imental stressors, with each being applied for 20 minutes and being separated from
¹⁹⁷ previous and subsequent stress exposures by ≥ 1 hour (similar to Rich & Romero
¹⁹⁸ 2005). Timing and type of experimental stressors were randomly selected each day
¹⁹⁹ to minimise the potential for habituation to each given stressor type. Experimen-
²⁰⁰ tal stressors included the presence of a novel object (a garden gnome), presence of
²⁰¹ a mock predator (a taxidermically mounted Cooper's hawk; *Accipiter cooperii*), cap-
²⁰² ture and restraint in an opaque fabric bag, presence of a human, covering of a given
²⁰³ flight enclosure with an opaque tarp (simulating extreme, inclement weather), and
²⁰⁴ presence of a taxidermically mounted conspecific fixed to the feeding platform of a
²⁰⁵ given flight enclosure (simulating a novel, dominant individual). In a previous study,
²⁰⁶ chickadees exposed to our randomised stressor protocol displayed a significant reduc-
²⁰⁷ tion in feeding rate and mass, and regular evoked alarm calls (Robertson *et al.* 2020b),
²⁰⁸ providing strong support for protocol efficacy. Endocrine responses to stressor types
²⁰⁹ were not measured to circumvent effects of blood sampling on surface temperature
²¹⁰ measurements and stress perception among sampled individuals. Individuals exposed
²¹¹ to control treatments were left undisturbed in an adjacent flight enclosure and blind
²¹² to experimenter presence.

213 Because flight enclosures were not auditorily segregated, estimated thermal responses
214 to stress exposure in this study (i.e., the interaction between time or ambient temper-
215 ature and treatment type) are expected to be conservative.

216 **2.3 | Infrared thermography, body surface temperature estima-**
217 **tion, and heat transfer estimation**

218 We monitored T_s and q_{Tot} of chickadees indirectly using remote infra-red thermogra-
219 phy (thermographic camera: FLIR VueProRTM, 13 mm, 226 × 356 resolution: accu-
220 racy = ± 5%; image frequency = 1 Hz), as per Robertson et al (2020a). Specifically, we
221 captured infrared thermographic images (radiometric JPEGs) of individuals at feeding
222 platforms across the duration of our experiment from weather-proofed camera boxes
223 mounted to the exterior of enclosure walls (0.5 m distance). To minimise temporal
224 bias of thermographic imaging among social groups, we rotated our thermographic
225 camera cardinally clock-wise among flight enclosures each day, with filming dura-
226 tions persisting for approximately one hour per enclosure, and the first flight enclosure
227 to receive thermographic filming being randomly selected on the morning of each
228 day. Because leg-ring combinations could not be readily distinguished from thermo-
229 graphic images, we also captured digital video (camera: Ion Air ProTM 1014W, 1280
230 × 720 resolution) of feeding individuals in parallel to themographic images to permit
231 individual identification. All thermographic imaging and digital video used in this
232 study were captured between 08:00h and 16:00h of each day.

233 Estimation of an object's T_s , and consequently rate of heat transfer by infrared ther-
234 mography requires that local ambient temperature and relative humidity are known
235 (Minkina & Dudzik, 2009; Tattersall, 2016). We therefore monitored ambient tem-

perature at enclosures subjected to thermographic filming using a ThermoChron iButton™ (Maxim Integrated, DS1922L-F5, San Jose, CA, USA) placed in the shade, at a frequency of 1 reading/5 minutes. Relative humidity readings were collected from a nearby weather station operated by Environment and Climate Change Canada (station identity = Hamilton A, 22 km from the experimental holding location) at the maximum available frequency of 1 reading/hour.

To estimate T_s from infrared thermographic images, we followed methods described by Robertson et al (2020a). Specifically, raw infra-red radiance (kW/m^2) values per pixel were manually extracted in R statistical software (version 3.6.1; R Core Team 2019) then first converted to temperature ($^\circ\text{C}$) per pixel according to Planck's law, ambient temperature and humidity estimates at the time of image capture, and equations outlined elsewhere (Minkina & Dudzik 2009; Tattersall 2016). Emissivity of the eye region of chickadees was assumed to be fixed at 0.95 according to estimates made for integument of Canadian and snow geese (*Branta canadensis* and *Chen caerulescens* respectively; Best & Fowler 1981). Following their estimation, temperature values per pixel were then integrated into FITS matrices using the R package FITSio (version 2.1.0; Harris 2016; one matrix per thermographic image), and eye region T_s values (here, maximum temperature values, as per Jerem *et al.* 2015) were manually extracted from within matrices using the open-sourced software FIJI (Schindelin *et al.* 2012; average size of eye region ≈ 230 pixels). To minimise underestimation of T_s as a consequence of image blurring, only values extracted from individuals that were stationary during image capture were included in our final data (Tattersall, 2016).

To estimate q_{Tot} (mW) from T_s measurements, we followed equations described by McCafferty *et al.* (2011) and Nord & Nilsson (2019). Here, however, values for the

kinematic viscosity of air (m^2/S ; at an assumed atmospheric pressure of 101.325 kPa) and the thermal expansion coefficient of air ($1/\text{K}$) were estimated for each given ambient temperature using the R packages "bigleaf" and "Thermimage" respectively (Knauer *et al.*, 2018; Tattersall, 2019). For this study, q_{Tot} was assumed to equal the sum of convective and radiative heat transfer, owing to both the minimal effects of wind-speed in our flight enclosures, and low likelihood of heat transfer between the eye region and any medium other than air during our experiment. Surface area of the eye region was estimated as 0.864 cm^2 according to Robertson *et al* (2020a), and contours within the eye region were considered negligible. Final q_{Tot} estimates were multiplied by two to represent total rates of heat transfer across both eye regions.

2.4 | Statistical analyses

All statistical analyses were conducted in R software (version 3.6.1; R Core Team 2019 with each generalised additive mixed-effects model ("GAMM") constructed in the package "brms" (version 2.13.3; Bürkner 2017). Additionally, all models were run using Markov Chain Monte Carlo (MCMC) sampling, with 4 Markov chains, 10000 chain iterations, and 1000 warm-up iterations to maximise mixing and convergence of Markov chains. Final iterations were thinned by 10 to account for possible autocorrelation between MCMC draws, and models were validated by visually diagnosing residual distributions and trace plots. \hat{R} values for all model parameters fell between 0.99 and 1.01, and the ratio of effective sample sizes to our total sample size were greater than 0.65 for each parameter. Lastly, all figures were produced in R using the package "ggplot2" (version 3.3.2; Wickham 2016), and one individual (a female captured in an urban environment) was removed due to an unusually small sample size ($n = 19$ thermographic images).

284 2.4.1 | Thermal responses to stress exposure among individuals

285 To first test whether acute and chronic changes in T_s accompanying the stress re-
286 sponses were repeatable among individuals, we constructed two Bayesian hierarchical
287 GAMMs wherein we estimated both global responses and individual-level responses
288 to stress exposure across acute and chronic time scales. In both models, tempera-
289 ture of the eye region of individuals ($^{\circ}\text{C}$; Gaussian distributed) was included as the
290 response variable, and treatment type (i.e. stress exposure or control) and sex were
291 included as linear, population-level predictors to account for the influence of each on
292 eye region temperature measurements. Additionally, flight enclosure identity, date of
293 thermographic image capture, and individual identity were included in each model as
294 group-level intercepts to account for statistical non-independence between measure-
295 ments collected from the same flight enclosure, day, and individual, and a group-level
296 slope for time of day per flight enclosure orientation (i.e. east facing or west facing)
297 was included to account for differential exposure to solar radiation within east- and
298 west-facing enclosures across time.

299 In our model predicting acute thermal responses to stress-exposure, time post stress
300 exposure (seconds), ambient temperature, and time of day (hour) were each included
301 as population level predictors. Because acute, stress-induced changes in T_s at the eye
302 region are thought to be non-linear (Jerem *et al.*, 2015, 2019), time post-stress ex-
303 posure was included as a cyclic cubic regression spline with 5 knots fixed at -1200,
304 0, 1200, 2400, and 3600 seconds to evenly distribute model fitting across each phase
305 of stress exposure (i.e. before, during, and after exposure). Here, a cyclic regression
306 spline was chosen to capture expected returns to baseline T_s (as reported for blue tits,
307 *Cyanistes caeruleus*; Jerem *et al.* 2019) following 40 minute recovery periods. To per-

308 mit comparisons between stress exposed and control treatments, we paired enclosures
309 such that time post stress exposure for an enclosure experiencing a control treatment
310 was considered to be equivalent to that of the nearest enclosure experiencing a stress
311 exposure treatment and equivalent cardinal orientation (i.e. west- or east- facing). As
312 such, our comparisons between treatments account for indirect effects of experimental
313 stress exposures on nearby control individuals.

314 In endotherms, T_s is expected to display non-linear relationships with both ambi-
315 ent temperature and time of day owing to peripheral thermoregulatory processes
316 (i.e. cold-induced vasoconstriction and warm-induced vasodilation) and circadian
317 rhythms (Richards, 1971; Cooper & Gessaman, 2005) respectively. Ambient tempera-
318 ture and time of day were therefore included as natural cubic and thinplate regression
319 splines respectively, each with 4 knots to minimise risk of model over-fitting. Knots
320 for our ambient temperature spline were evenly spaced by quantiles to uniformly cap-
321 ture trends in eye region temperature at ambient temperatures below, within, and
322 above thermoneutrality for our study species (Grossman & West, 1977). Because we
323 did not have *a priori* assumptions for knot positions for our time of day spline, knot
324 positions were chosen by truncated eigen decomposition (Wood, 2003). To control
325 for differential effects of treatment type on T_s across time (Jerem *et al.*, 2015, 2019) and
326 ambient temperature (Robertson *et al.* 2020a), population-level interactions between
327 treatment type and ambient temperature, and treatment type and post stress exposure
328 were also included as model predictors, along with an interaction between treatment
329 type and the tensor product (\otimes) between ambient temperature and time post stress ex-
330 posure to account for the influence of ambient temperature on acute thermal responses
331 to stress exposure at the skin (Nord & Folkow, 2019). All interaction terms were pe-
332 nalised on the first derivative to minimise the potential for concurvity between inter-

333 action terms and main effects. Finally, to estimate differences in acute, stress-induced
334 changes in T_s among individuals, group-level slopes for time post stress exposure and
335 the interaction between time post-stress exposure and treatment type were included
336 for each individual. Correlation between adjacent T_s measurements was corrected
337 using a type-I autoregressive (AR1) correlation structure with an estimated rho (ρ) of
338 0.69, and residual error was estimated independently for each treatment type.

339 In our model predicting chronic stress-induced changes in T_s , group-level predictors
340 remained as described above but with minor adjustments. Specifically, all predictors
341 including time post stress exposure (i.e. as a main effect or interactive effective) were
342 excluded from our model to permit assessment of long-term, but not short-term trends
343 in T_s according to treatment type. Furthermore, to estimate differences in chronic
344 stress-induced changes in T_s among individuals, group-level slopes for ambient tem-
345 perature and the interaction between ambient temperature and treatment type was
346 included per individual. Here, ambient temperature was mean-centered and scaled to
347 2 times the standard deviation (as per Araya-Ajoy *et al.* 2015) to allow for individual
348 slopes to be estimated with respect to our average environmental conditions. Again,
349 correlations between adjacent T_s measurements was corrected using an AR1 corre-
350 lation structure ($\rho = 0.69$), and residual error was estimated separately per treatment.

351

352 Because rates of peripheral heat transfer (q_{Tot}) are proportional to T_s at given ambient
353 temperatures, both acute and chronic changes in q_{Tot} accompanying stress exposure
354 treatments were modeled as described above. In these models, however, q_{Tot} was used
355 as the response variable (mW; Gaussian distributed) in place of T_s .

356 In all hierarchical models, we used informed priors for our population intercept, our
357 coefficients for treatment (linear), sex (linear), ambient temperature (first order, linear),
358 and our values for spline smoothness (ϕ), with prior distributions being informed by
359 Robertson et al (2020a). For our model intercepts, we used gamma distributed priors
360 with α values of 60 and 50 (T_s models and q_{Tot} models respectively), and β values of
361 2 (both T_s models and q_{Tot} models) thus assuming positive T_s and q_{Tot} values at an
362 ambient temperature of 0°C, with peak densities of approximately 30°C and 25 mW
363 respectively. In all models, priors for treatment type and sex were normally distributed
364 with means of 0 and -1 respectively, and standard deviations of 2.5, whilst those for ϕ
365 were gamma distributed with $\alpha = 2$, and $\beta = 0.5$ owing to low expected "wigginess"
366 in our smooth terms. Lastly, for our first order slope of ambient temperature, we used
367 gamma distributed priors ($\alpha = 4$, $\beta = 2$) in our models pertaining to T_s and normally
368 distributed priors (mean = -5, s.d. = 5) in our models pertaining to q_{Tot} because the
369 relationship between ambient temperature and T_s is expected to be positive, whilst
370 that between ambient temperature and q_{Tot} is expected to be negative (Robertson *et al.*
371 2020b). Uninformative priors were used for all other model parameters; specifically,
372 priors for the standard deviation of population level and group level predictors followed
373 student's t distributions with 3 degree of freedom, location parameters of 0 and a
374 scale factors of 3.4. Similarly, priors for sigma parameters also followed student's t
375 distributions with 3 degrees of freedom and location parameters of 0, however, scale
376 factors were reduced to 2.5.

377 2.4.2 | Repeatability estimates

378 To calculate repeatability of stress-induced changes in T_s and q_{Tot} , we followed meth-
379 ods described by Araya-Ajoy *et al.* (2015). Their methods, however, are largely de-
380 scriptive and do not test the presence or absence of trait repeatability within an ex-

381 experimental context. To correct for this, we constructed null models (i.e. models with
382 individual identities scrambled) for T_s and q_{Tot} across both acute and chronic time-
383 periods, then compared mean repeatability estimates (per Markov chain iteration) ac-
384 quired from true and null model posterior distributions. Here, a significant increase
385 in repeatability values derived from true models relative to those derived from null
386 models suggests that true repeatability values could not be explained by biases in the
387 experimental process alone. Null models were constructed by randomly allocating in-
388 dividual identities to each heat transfer estimate, then re-running hierarchical models
389 as described above (Figure 2). To control for possible effects of treatment order during
390 identity randomisation, we limited possible identity assignments to individuals that
391 had experienced the same treatment order as the true individual from which the T_s
392 or q_{Tot} was obtained. Mean repeatability estimates were then compared between our
393 true and null models using two, one-way, non-linear hypothesis tests in the R package
394 "brms" (Bürkner, 2017). For all hypothesis tests, priors for true and null repeatability
395 estimates were beta distributed with peaks at 0 ($\alpha = 1$, and $\beta = 4$). Bayes factors (K),
396 representing support for true repeatability estimates being greater than null repeata-
397 bility estimates, were calculated from each hypothesis test using the Savage-Dickey
398 density ratio method (Wagenmakers *et al.*, 2010).

399 2.4.3 | Effects of stress exposure on repeatability estimates

400 Traits under directional or stabilising selection are thought to display lower variability
401 than those that are selectively neutral (e.g. Gibson & Bradley 1974; Lande & Arnold
402 1983; Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001). Furthermore, the po-
403 tential for traits to respond to selection is contingent upon trait expression being con-
404 sistent across time (e.g. repeatable; Dochtermann *et al.* 2015; but see Dohm 2002).
405 Thus, the presence of both high repeatability (R) and relatively low residual varia-

tion (" ϵ " in a linear or additive model) is suggestive of previous or current selection acting upon a trait's expression, if all other environmental variables and sources of measurement error are controlled (i.e wherein ϵ is the sum of residual variation explained by external environmental factors, measurement error, and within-individual variability; suggestive in Gibson & Bradley 1974 and Boake 1989). In our experiment, both stress-exposed and control individuals experienced the same environmental conditions, and measurement error around T_s and q_{Tot} was unlikely to differ systematically between stress-exposed and control treatments. Thus, to test for evidence of enhanced stabilising or directional selection (past or current) on the expression of T_s and q_{Tot} during stress exposure relative to resting conditions, we compared error and repeatability estimates obtained for stress-exposed and control treatments across both short and long-term time-frames (e.g. acute and chronic, respectively). To do so, both error and repeatability estimates drawn from posterior distributions of acute and chronic models (pertaining to both T_s and q_{Tot} ; described above) were compared using one-way, non-linear hypothesis tests as described previously (subsection "Repeatability estimates"). Priors for repeatability and error estimates under control and stress-exposed conditions were beta ($\alpha = 1$; $\beta = 4$) and normally distributed (mean = 0, s.d. = 0.25) respectively. Again, Bayes factors were calculated for each test using the Savage-Dickey density ratio method (Wagenmakers *et al.*, 2010), with results representing relative support for either decreased error or increased repeatability within stress exposure treatments when compared with control treatments.

2.4.4 | Effects of urbanisation on stress-induced thermal responses

To test whether flexible changes in T_s and q_{Tot} accompanying acute stress exposures differed between urban and rural chickadees, we first extracted mean coefficients for the interactions between treatment type and time post stress exposure for each individual.

⁴³¹ vidual from the posterior distribution of our acute models. Mean coefficients were
⁴³² then compared between capture ecotypes using Bayesian "ANOVAs" in the R pack-
⁴³³ age "BayesFactor" (version 0.9.12.4.2; Morey *et al.* 2019) with capture location (one
⁴³⁴ of six) included as a group-level intercept. To test whether chronic changes in T_s
⁴³⁵ and q_{Tot} following stress exposures differed between individuals from urban and rural
⁴³⁶ locations, we used a similar approach, however, mean coefficients for the interactions
⁴³⁷ between ambient temperature and treatment type were extracted from posterior dis-
⁴³⁸ tributions and used as response values. Priors for the effect of capture ecotype and
⁴³⁹ capture location on individual slopes were weak and Cauchy distributed with scale
⁴⁴⁰ parameters of $2^{1/2}$ and 1 respectively, whilst Jeffreys priors were used for our intercept
⁴⁴¹ and residual error term (τ) (Rouder *et al.*, 2012).

⁴⁴² 3 | RESULTS

⁴⁴³ Credible intervals (95%) are reported for model coefficients in crotchets. All reported
⁴⁴⁴ means are marginal and are given \pm one standard deviation (s.d.).

⁴⁴⁵ 3.1 | Stress-induced changes in body surface temperature and ⁴⁴⁶ peripheral heat loss are repeatable

⁴⁴⁷ Our analyses detected rapid and pronounced changes in both eye region tempera-
⁴⁴⁸ ture (" T_s ") and heat loss from the eye region (" q_{Tot} ") of chickadees following stress
⁴⁴⁹ exposure " T_s : $\beta = 1.68 [0.36, 4.58]$; q_{Tot} : $\beta = 2.79 [0.86, 6.90]$ "; Table 1). Similar and
⁴⁵⁰ simultaneous changes in T_s and q_{Tot} were not detected in nearby control individuals
⁴⁵¹ (Table 1). Interestingly, the magnitude and direction of stress-induced T_s and q_{Tot}
⁴⁵² responses were dependent upon ambient temperature (T_s : $\phi = 4.85 [0.62, 10.90]$; q_{Tot} :

⁴⁵³ $\phi = 6.58 [0.64, 15.50]$; Table 1). Specifically, at low ambient temperatures (i.e. those
⁴⁵⁴ below thermoneutrality; $< 14^{\circ}\text{C}$), individuals exposed to stressors displayed rapid and
⁴⁵⁵ transient increases in T_s and q_{Tot} , with elevations in T_s and q_{Tot} persisting for approx-
⁴⁵⁶ imately 30 minutes (1800 seconds) after stressor completion (Figures 3a and 3b). At
⁴⁵⁷ our lowest observed ambient temperature (3°C), T_s among stress-exposed individuals
⁴⁵⁸ increased by an average of $5.53^{\circ}\text{C} \pm 0.154^{\circ}\text{C}$ (with respect to baseline measurements)
⁴⁵⁹ immediately upon stressor completion (Figure 3a), and this increase corresponded to
⁴⁶⁰ a rise in q_{Tot} of $11.50 \pm 0.24 \text{ mW}$ (Figure 3b). In contrast, at high ambient temper-
⁴⁶¹ atures (i.e. those above thermoneutrality; $> 30^{\circ}\text{C}$), an inverted response among stress
⁴⁶² exposed individuals was detected, with individuals displaying rapid and transient re-
⁴⁶³ ductions in T_s and q_{Tot} (Figures 3a and 3b) in response to stress exposures (albeit small).
⁴⁶⁴ At these ambient temperatures, decreases in T_s and q_{Tot} persisted for approximately
⁴⁶⁵ 20 minutes (1200 seconds) following stressor completion, with mean T_s and q_{Tot} de-
⁴⁶⁶ creasing by approximately $1.15^{\circ}\text{C} \pm 0.152^{\circ}\text{C}$ and $2.23 \pm 0.24 \text{ mW}$ respectively at
⁴⁶⁷ our highest observed ambient temperature (38.5°C ; again, with respect to baseline
⁴⁶⁸ measurements) upon stressor completion (Figures 3a and 3b). A small effect of time
⁴⁶⁹ post stress exposure on both T_s and q_{Tot} among control individuals was detected (ϕ
⁴⁷⁰ $= 0.45 [0.03, 1.90]$; Table 1), however, neither increases nor decreases in T_s and q_{Tot}
⁴⁷¹ were detectable following onset of stress exposures (here, in the nearest-by flight en-
⁴⁷² closures designated for stress exposure treatments) above or below the thermoneutral
⁴⁷³ zone (Figure 3b). Neither T_s nor q_{Tot} differed between sexes (T_s : $\beta_{\text{Sex}} = -0.08 [-0.46,$
⁴⁷⁴ $0.33]$; q_{Tot} : $\beta_{\text{Sex}} = -0.17 [-0.86, 0.49]$; Table 1), and treatment type alone did not in-
⁴⁷⁵ fluence each measure (T_s : $\beta_{\text{Treatment}} = 0.26 [-0.19, 0.99]$; q_{Tot} : $\beta_{\text{Treatment}} = 0.29 [-0.28,$
⁴⁷⁶ $1.25]$; Table 1).

⁴⁷⁷ Beyond the acute responses, our analyses also detected chronic effects of stress expo-

478 sures on T_s and q_{Tot} across our sample population (T_s model: $\beta = 1.81$, [0.32, 5.58];
479 q_{Tot} model: $\beta = 2.51$, [0.61, 6.92]; Table 2). Specifically, both T_s and q_{Tot} of stress-
480 exposed individuals decreased at low ambient temperatures and increased at high am-
481 bient temperatures relative to controls (Table 2; Figure 4). On average, T_s was 1.89°C
482 $\pm 1.22^\circ\text{C}$ lower in stress-exposed individuals than control individuals at our lowest
483 observed ambient temperature, and $1.64^\circ\text{C} \pm 0.95^\circ\text{C}$ higher in stress-exposed indi-
484 viduals than control individuals at our highest observed ambient temperature. Such
485 trends in T_s corresponded to reductions in q_{Tot} of approximately $3.75 \pm 2.56 \text{ mW}$
486 at our lowest observed ambient temperature, and increases in q_{Tot} of approximately
487 $2.56 \pm 1.99 \text{ mW}$ at our highest observed ambient temperature among stress exposed
488 individuals relative to controls (Figure 4). Similar to our results pertaining to acute
489 thermal responses, neither T_s nor q_{Tot} differed between sexes in our chronic model
490 (T_s : $\beta_{Sex} = 0.02$ [-0.41, 0.44]; q_{Tot} model: $\beta_{Sex} = 0.03$ [-0.71, 0.76]; Table 2) and no
491 effect of treatment alone on T_s or q_{Tot} was detected (T_s : $\beta_{Treatment} = 0.02$ [-0.16, 0.20];
492 q_{Tot} : $\beta_{Treatment} = 0.00$ [-0.29, 0.29]).

493 As predicted, acute stress-induced changes in T_s and q_{Tot} were significantly repeat-
494 able among chickadees. Namely, repeatability values calculated from our true models
495 exceeded those calculated from our null model (i.e. with individual identities scram-
496 bled; non-linear hypothesis test: $K_{T_s} > 100$; $K_{q_{Tot}} = 47.00$; Figure 5a and SFigure
497 5a), suggesting that repeatability of acute thermal responses to stress exposure not
498 only exceeded zero, but also could not be explained by biases in our experimental
499 methodology. Nevertheless, the degree to which these acute thermal responses were
500 repeatable among chickadees was low (surface temperature [T_s]: $R_{\text{stress exposure}} = 0.14$
501 [0.03, 0.32]; heat transfer [q_{Tot}]: $R_{\text{stress exposure}} = 0.11$ [0.02, 0.27]; Table 1), suggesting
502 that whilst some variation in acute thermal responses is probably attributable to con-

503 sistent differences in stress-responsive phenotypes among individuals, the majority of
504 such variation is perhaps better explained by other sources of variation (e.g. environ-
505 mental or measurement). Similar to acute changes in T_s and q_{Tot} , chronic changes in
506 T_s and q_{Tot} following stress exposure (or "chronic reaction norms") were significantly
507 repeatable among chickadees. Again, repeatability values estimated from our true
508 models exceeded those estimated from our null models, suggesting that repeatability
509 of chronic changes in T_s and q_{Tot} observed in our study were unlikely to be explained
510 by biases in our experimental method (non-linear hypothesis tests comparing true and
511 null models; $K > 100$ for both T_s and q_{Tot} ; Figure 5b and SFigure 5b). Here, however,
512 repeatability of chronic reaction norms among chickadees was high ($R_{T_s} = 0.61 [0.35,$
513 $0.81]$; $R_{q_{Tot}} = 0.67 [0.44, 0.84]$; Table 2), indicating that stress-induced changes in T_s
514 and q_{Tot} consistently varied among individuals.

515 3.2 | Evidence for stabilising or directional selection on stress- 516 induced changes in body surface temperature and periph- 517 eral heat loss

518 Across acute time-periods (i.e. ≤ 1 hour), T_s of control individuals was significantly
519 more variable and less consistent than that of stress-exposed individuals, after control-
520 ling for circadian rhythms and environmental effects (e.g. ambient temperature, solar
521 radiation; $\sigma_{Control} = 1.21 [1.19, 1.24]$, $\sigma_{Stress} = 1.18 [1.14, 1.22]$; $R_{control} = 0.07 [0.01,$
522 $0.18]$, $R_{stress \ exposure} = 0.14 [0.03, 0.32]$; Table 4.1). As predicted, these difference in vari-
523 ance and repeatability between treatments were strongly and moderately supported
524 by non-linear hypothesis tests respectively ($K_{variance} = 72.47$; $K_{repeatability} = 6.66$; SFigure
525 6). Similarly, q_{Tot} at the eye region of chickadees was both slightly less variable and
526 more repeatable during stress exposure treatments than control treatments ($\sigma_{Control} =$
527 $2.09 [2.06, 2.13]$, $\sigma_{Stress} = 2.06 [1.99, 2.12]$; $R_{control} = 0.06 [0.01, 0.16]$, $R_{stress \ exposure} =$

528 0.11 [0.02, 0.27]; Table 1). These differences in unexplained variability and repeatabil-
529 ity, however, were only moderately and weakly supported by non-linear hypothesis
530 tests respectively ($K_{\text{variance}} = 10.65$; $K_{\text{repeatability}} = 5.24$; SFigure 7).

531 Similar to acute time periods, T_s of chickadees was more variable and less repeatable in
532 control treatments than in stress exposure treatments across chronic time periods (i.e.
533 ≤ 30 days), after controlling for circadian and environmental effects ($\sigma_{\text{Control}} = 1.20$
534 [1.18, 1.23], $\sigma_{\text{Stress}} = 1.17$ [1.13, 1.21]; $R_{\text{control}} = 0.34$ [0.17, 0.56], $R_{\text{stress exposure}} = 0.61$
535 [0.35, 0.81]; Table 2). Again, as predicted, these differences in variance and repeata-
536 bility were strongly and moderately supported by respective non-linear hypothesis
537 tests ($K_{\text{variance}} = 48.32$; $K_{\text{repeatability}} = 15.51$; SFigure 8). Variability and repeatability
538 of q_{Tot} across chronic time periods followed similar patterns, with variability again
539 being lower and repeatability again being higher in stress-exposed chickadees, when
540 compared with rested (i.e. control) chickadees ($\sigma_{\text{Control}} = 2.07$ [2.04, 2.11], $\sigma_{\text{Stress}} =$
541 2.04 [1.98, 2.10]; $R_{\text{control}} = 0.41$ [0.22, 0.63], $R_{\text{stress exposure}} = 0.67$ [0.44, 0.84]; Table 2).
542 These differences were moderately and strongly supported by non-linear hypothesis
543 tests respectively ($K_{\text{variance}} = 9.62$; $K_{\text{repeatability}} = 16.73$; SFigure 9), as predicted.

544 3.3 | Stress-induced thermal responses do not differ between 545 urban and rural individuals

546 The magnitude of acute changes in T_s or q_{Tot} (or "acute reaction norms") following
547 stress exposure did not differ between chickadees captured from urban or rural eco-
548 types (T_s : $\mu_{1:\hat{u}rban} = 0.09$ [-0.25, 0.82]; $\mu_{1:\hat{r}ural} = 0.14$ [-0.23, 0.54]; q_{Tot} : $\mu_{1:\hat{u}rban} = 0.19$
549 [-0.35, 0.87]; $\mu_{1:\hat{r}ural} = 0.14$ [-0.34, 1.28]; $n = 9$ urban, $n = 10$ rural; Figure 6; SFigure
550 10). Indeed, ANOVAs including capture ecotype as a population-level predictor were

551 less likely to explain the magnitude of T_s or q_{Tot} responses among individuals than
552 ANOVAs without (T_s : $K = 0.25$; q_{Tot} : $K = 0.24$). Similar results were detected at the
553 chronic level, with the magnitude of chronic stress-induced changes in T_s and q_{Tot} (or,
554 "chronic reaction norms") remaining similar between urban- and rural-origin chick-
555 adees (T_s : $\mu_{1:urban} = -0.25$ [-4.20, 1.88]; $\mu_{1:rural}$; q_{Tot} : $\mu_{1:urban} = -0.25$ [-4.20, 1.88];
556 $\mu_{1:rural} = 0.20$ [-3.68, 5.89]; $n = 9$ urban, $n = 10$ rural; Figure 6). Again, ANOVAs
557 including capture ecotype as a predictor were less likely to explain the magnitude of
558 chronic changes in T_s and q_{Tot} than ANOVAs without (T_s : 0.24; q_{Tot} : 0.25).

559 4 | DISCUSSION

560 4.1 | Acute and chronic thermal responses to stress exposure are 561 repeatable

562 Our results show that flexible changes in surface temperature (T_s) and rate of heat
563 transfer (q_{Tot}) following stress exposures are repeatable in chickadees, whether ob-
564 served across acute or protracted (i.e. chronic) time periods. Such repeatability fulfills
565 a critical first prediction of the hypothesis that stress-induced flexibility of T_s and q_{Tot}
566 may experience evolutionary responses to selection. Notably, however, the extent to
567 which flexibility of T_s and q_{Tot} was repeatable appeared to depend upon the time pe-
568 riod of observation (Figure 5 and SFigure 5). Across acute time periods, the shape and
569 magnitude of stress-induced T_s and q_{Tot} responses were appreciably similar among
570 individuals (Figures 3b and 5a; SFigure 5a). Across chronic time-periods, however, a
571 considerably wider range of stress-responsive phenotypes among individuals emerged
572 (Figures 4 and 5b; SFigure 5b). To our knowledge, our study is the first to report re-
573 peatability of stress-induced flexibility of T_s and q_{Tot} in any vertebrate.

574 The high degree with which chronic responses to stress exposure varied among our

study individuals highlights that, despite a clear average trend among individuals (Figure 4; Table 1), reductions in average T_s and q_{Tot} in the cold and increases in average T_s and q_{Tot} in the warmth are clearly not generalisable responses to repeated stress perception in birds. Among some individuals, for example, repeated stress exposure appeared to elicit the reverse response, with mean T_s and q_{Tot} rising in the cold and decreasing in the heat (Figure 4). If the emergence of such chronic stress-induced responses are largely fixed within individuals, as our study suggests, theorised energetic benefits ascribed to this response (e.g. Robertson *et al.* 2020b) may only be accrued by some and not all individuals. Given that survivorship has been linked to efficiency of energy use in extreme and challenging environments (Parsons, 2005), such discrepancies in theorised energetic savings could provide opportunities for selection to act upon chronic thermal responses to stress exposure in our study species.

Any evolutionary responses to selection on flexibility of T_s and q_{Tot} in response to chronic stress exposures requires that this trait is underpinned by heritable genetic architecture. In this study, we chose to monitor changes in T_s and peripheral q_{Tot} in response to stress exposure alone. Therefore, whether chronic responses observed here emerge as a consequence of stress-induced changes in core body temperature, peripheral temperature (e.g. by changes in vascular flow; Oka *et al.* 2001), or both remains unknown. Regardless of their anatomical origin, the possibility of individual differences in chronic responses arising from differences in genetic architecture is well supported. At the level of core tissues, for example, both heterothermy and facultative hypothermia appear phylogenetically constrained (Boyles *et al.*, 2013; Gerson *et al.*, 2019), and recent studies in poultry have provided strong evidence for the direct influence of genetic polymorphisms and differential gene transcription on heat dissipation capacity and the magnitude of core body temperature increases in supra-

thermoneutral ambient temperatures (Srikanth *et al.*, 2019; Zhuang *et al.*, 2019). Similarly, at the level of the periphery, studies in humans have elucidated several genetic polymorphisms that appear to dictate the duration and magnitude of peripheral vascular responses to cold and psychological stress (e.g. Rao *et al.* 2008; Chen *et al.* 2010; Kelsey *et al.* 2010, 2012; Huang *et al.* 2012) that could have meaningful consequences on environmental heat transfer; many such polymorphisms correspond to genes with conserved functions among tetrapods (Vincent *et al.* 1998; Yamamoto & Vernier 2011; Céspedes *et al.* 2017; Dopamine β -hydroxylase in sauropsids: Lovell *et al.* 2015). Consequently, variation in stress-induced changes in T_s and q_{Tot} among our chickadees may well be heritable, regardless of whether such responses are driven by changes in thermogenesis at the core, or by changes in peripheral vascular flow and consequential changes in environmental heat transfer (Robertson *et al.* 2020a).

Still, we cannot refute the possibility that our observed chronic responses to stress exposure are broadly labile within individuals and dictated by energetic or resource constraints that were not measured here. For example, Robertson *et al* (2020b) recently argued that stress-induced changes in T_s and q_{Tot} may be understood as trade-offs that are predominantly manifested under negative energetic balance. It is possible that our experimental conditions may have contributed to fixed and non-random resource allocation among individuals (e.g. via dominance interactions; Ratcliffe *et al.* 2007) that dictated how stress-induced thermal responses at the eye region emerged. In such a case, any evolutionary responses to selection on these responses may better reflect patterns of resource monitoring and allocation during a challenge, rather than fixed reflexes within individuals. Experiments seeking to tease apart the influence of resource availability and fixed individual variation on chronic thermal responses to stress exposure are therefore warranted.

625 To our surprise, the degree to which individuals acutely shifted their T_s and q_{Tot} in
626 response to stress exposures displayed considerable overlap (Figures 3a and 3b). Such
627 overlap among individuals, coupled with the significant predictive effects of other en-
628 vironmental parameters (e.g. ambient temperature and time of day; Table 1) implies
629 that, unlike chronic thermal responses, the manifestation of acute thermal responses
630 to stress exposure is perhaps better explained by the combination of common trait
631 expression and environmental effects than variation in intrinsic factors among indi-
632 viduals. In domestic rats (*Rattus norvegicus domestica*), ambient temperature has been
633 shown to strongly influence the magnitude of acute changes in core body tempera-
634 ture, with responses typically being largest at low ambient temperature and smallest
635 at high ambient temperatures (Briese 1992; reviewed in Oka 2018). Similarly, in
636 Svalbard rock ptarmigans (*Lagopus muta hyperborea*), the magnitude of stress-induced
637 changes in skin temperature are reportedly larger at low ambient temperature than
638 at comparatively higher ambient temperatures (Nord & Folkow, 2019). As such, the
639 emergence of acute, stress-induced changes in T_s and q_{Tot} in our sample population
640 may have been largely dictated by modulatory effects of ambient temperature alone,
641 with little remaining variation explained by phenotypic differences among individ-
642 uals. In any case, the relatively low repeatability of acute stress-induced thermal re-
643 sponds (observed here) highlights that the potential for this response to respond to
644 selection in black-capped chickadees is probably low.

645 4.2 | Variation in eye region temperature and heat loss is re- 646 duced during stress exposure

647 Interestingly, unexplained variation in both T_s and q_{Tot} was higher during control
648 treatments than during stress exposure treatments (Tables 1-2). Additionally, both T_s

and q_{Tot} were more repeatable during stress exposure treatments than control treatments (SFigures 6–9) regardless of the time period of observation (i.e. \leq 1 hour, or \leq 30 days). Together, these trends indicate that either: 1) T_s and q_{Tot} are more tightly regulated during stress exposures than during resting conditions, or 2) T_s regulation is relaxed during stress exposures, thereby allowing T_s to conform to ambient temperatures (as observed in other avian species; reviewed in Angilletta *et al.* 2019). Regardless of the mechanism, the relative consistency with which T_s and q_{Tot} emerge during stress exposures suggests that their maintenance has, perhaps, experienced stronger directional or stabilising selection than that during rested (i.e. control) conditions (our second prediction; e.g. Gibson & Bradley 1974; Lande & Arnold 1983; Van Homrigh *et al.* 2007; but see Kotiaho *et al.* 2001). Such findings lend credence to a critical role of heat-transfer regulation during stress exposure, that, to our knowledge, has received little to no research attention.

When contextualised with variability within other stress-physiological processes, reduced variability of T_s and q_{Tot} during stress perception is perhaps not unusual. Variability in heart rate is widely known to fall during stress exposure in many vertebrate species (e.g. Visser *et al.* 2002; Von Borell *et al.* 2007; Cyr *et al.* 2009). Similarly, within-individual variation in stress-induced glucocorticoid production has been reported to be lower than that of baseline production in both avian and amphibian species (e.g. Cockrem & Silverin 2002; Rensel & Schoech 2011; Narayan *et al.* 2012; Grace & Anderson 2014; but see Narayan *et al.* 2013; Baugh *et al.* 2014; Lendvai *et al.* 2015). Such trends indicate that the collective traits enabling individuals to conform or cope with environmental challenges (together, the "stress phenotype") have experienced strong directional or stabilising selection (Ellis *et al.*, 2006). Modulation of T_s and q_{Tot} during stress exposure (whether by a reduction or increase) may, therefore,

simply represent a little-discussed constituent of the vertebrate stress phenotype that contributes to successful coping. Although the ultimate value of stress-induced T_s and q_{Tot} modulation is unclear, the bivalent nature, ambient-temperature dependence, and direct implications on energetic savings in our study (albeit small; Figure 4) triangulate on a relaxation of expenditure toward thermoregulation (the Thermoprotective Hypothesis; Robertson *et al.* 2020a). On the other hand, rapid increases in T_s and q_{Tot} at low ambient temperatures, and rapid declines in T_s and q_{Tot} following stress exposure (as observed here; Figures 3a and 3b) may suggest that at the acute level, changes in T_s occur to promote enzymatic, neuronal, or muscular function during the stress responses (i.e. owing to Q10 effects: e.g. Carr & Lima 2013), rather than to reduce thermoregulatory expenses.

4.3 | Urban and rural individual do not differ in stress-induced thermal responses

In sharp contrast to our predictions, the degree to which T_s and q_{Tot} flexibly responded to acute or chronic stress exposure did not differ between chickadees captured from urban and rural environments (Figure 6 and SFigure 10). According to our results, individuals from urban environments appear no more able to flexibly shift their T_s and thermoregulatory expenditure during stress exposure than those from rural environments. We propose four possible explanations for these findings. First, insufficient generations spent within a given ecotype may have limited opportunities for evolutionary responses to selection on stress-induced thermal responses to occur in our study species. The combination of low juvenile dispersal, high site fidelity among adults (Weise & Meyer, 1979), and relatively short generation time in our study species, however, suggests that this is unlikely (reviewed in McDonnell & Hahs 2015). Furthermore, genetic differentiation between individuals captured in urban and rural

environments has recently been reported for a closely related Parid species (the great tit, *Parus major*; Perrier *et al.* 2018), supporting the possibility of responses to selection imposed by urban environments. A second, and arguably more likely explanation for our findings is that costs of urban living in chickadees are no higher than those of rural living, despite a theoretically increased frequency in stress exposure events. Although direct comparative field studies are lacking (Sepp *et al.*, 2018), trends in basal metabolic rate of another temperate bird species (the house finch, *Haemorhous mexicanus*) do suggest that energetic expenditure may not differ between individuals captured from urban and rural environments (at least, at rest: Hutton *et al.* 2018). In chickadees, urban environments may afford opportunities to access novel and abundant food sources (Robb *et al.*, 2008; Prasher *et al.*, 2019) that could offset energetic costs associated with frequent activation of emergency pathways (but see Demeyrier *et al.* 2017). Strategies to relax expenditure towards other biological process (e.g. thermoregulation), therefore, may be no more likely to emerge in urban population than rural populations. Third, the degree of urbanisation in our selected urban and rural locations may not have differed sufficiently to impose differential patterns of selection (but see Appendix). Given our low sample size, assessing linear correlations between the degree of urbanisation at capture locations and the magnitude of stress-induced thermal responses was unfortunately not possible. Future studies assessing these response among individuals from more urbanised locations may be warranted. Lastly, neither acute nor chronic changes in T_s and q_{Tot} that accompany stress exposures may be heritable in chickadees. Previous studies, both within and across species, have suggested that changes in core body temperature and peripheral vascular flow during a challenge are underpinned by heritable genetic architecture (discussed above). Nevertheless, it is indeed possible that thermal responses to stress exposure at either the acute or chronic level are merely contingent upon environmental context (e.g. resource availability) and

725 the maximum degree to which T_s and q_{Tot} can flexibly respond to stress exposure is
726 fixed among individuals. Further studies questioning the heritability of stress-induced
727 thermal responses in this species are, therefore, critical to understanding whether this
728 response may provide opportunities to adapt to a warming and urbanising world.

729 4.4 | Summary

730 Recent empirical studies have argued that endotherms may balance costs associated
731 with responding to perceived stressors by flexibly decreasing their T_s and q_{Tot} in the
732 cold, and flexibly increasing their T_s and q_{Tot} in warmth. By doing so, energy may
733 be allocated away from costly thermogenesis or evaporative cooling, and toward the
734 immediate demands of coping with the challenge at hand (Robertson *et al.* 2020a). In
735 chickadees, we tested whether such stress-induced flexibilities of T_s and q_{Tot} are re-
736 peatable among individuals and thus offer opportunities for endotherms to cope with
737 costs that typify urbanised environments, across generations. As predicted, we show
738 that both acute and chronic changes in T_s and q_{Tot} during stress exposure are repeat-
739 able, however, only those at the chronic level displayed meaningfully high repeatabil-
740 ity estimates (T_s : $R_{chronic} = 0.61$; q_{Tot} : $R_{chronic} = 0.67$). Furthermore, we show that both
741 T_s and q_{Tot} are less variable within individuals, and more variable among individuals
742 during experimental stress exposure than during control treatment, suggesting that
743 regulation of T_s and q_{Tot} during the stress response has probably experienced direc-
744 tional or stabilising selection. Both trends, to our knowledge, are yet to be reported
745 in any vertebrate. To our surprise, neither acute, nor chronic flexibility of T_s and q_{Tot}
746 in response to stress exposure differed between urban- and rural-origin chickadees.
747 Together, our results suggest that whilst flexibility of T_s and q_{Tot} meet a critical first
748 criterion for responsiveness to selection and may enhance energetic efficiency of some

⁷⁴⁹ but not all individuals, those residing in urban environments are no more likely to
⁷⁵⁰ acquire benefits associated with this flexibility than those in rural environments.

Tables

TABLE 1 Acute effects of stress exposure on eye region temperature (T_s) and dry heat transfer (q_{Tot}) of black-capped chickadees ($n = 19$; $n = 9$ females, $n = 10$ males); results of two hierarchical GAMMs. Obelisks (\dagger) represent smooth terms, for which estimates refer to the degree of smoothness (ϕ : 0 = linear slope). Estimates for remaining population-level terms represent linear slopes, whilst those for group-level effects represent standard deviations. Degree of smoothness and 95% credible intervals ("CIs") for tensor products represent means across penalisation groupings, and effective sample sizes represent sums across groupings. Eye region temperature measurements were estimated from infrared thermographic images ($n = 5599$) captured across 60 days. T_s model: $R^2 = 0.85$; q_{Tot} model: $R^2 = 0.94$. Asterisks (*) represent statistically significant terms (95% credible intervals do not cross zero).

Population-level Predictors			
Term	T _s Estimate [95% CIs]	q _{Tot} Estimate [95% CIs]	Effective Sample Size (T _s /q _{Tot})
Intercept*	33.09 [30.84, 35.10]	19.02 [15.16, 25.30]	3644/3600
Treatment	0.26 [-0.19, 0.99]	0.29 [-0.28, 1.25]	3726/3917
Sex (Male)	-0.08 [-0.46, 0.33]	-0.17 [-0.86, 0.49]	3536/3714
†Ambient Temperature*	1.63 [0.38, 5.03]	1.38 [0.16, 5.00]	3537/3680
†Ambient Temperature: Treatment*	1.47 [0.19, 5.07]	1.87 [0.29, 5.74]	2870/3191
†Time Post Stress Exposure*	0.45 [0.03, 1.90]	0.65 [0.05, 2.52]	3273/3440
†Time Post Stress Exposure: Treatment*	1.68 [0.36, 4.58]	2.79 [0.86, 6.90]	3566/3679
†[Time Post Stress Exposure \otimes Ambient Temperature]: Treatment*	4.85 [0.62, 10.90]	6.58 [0.64, 15.50]	10141/10674
†Hour \otimes Orientation*	3.68 [0.65, 10.10]	4.19 [0.64, 10.70]	10571/10674
Group-level Predictors			
Bird Identity	0.32 [0.20, 0.50]	0.56 [0.35, 0.87]	3763/3121
Date of Photo	1.79 [1.37, 2.32]	3.30 [2.50, 4.20]	3254/3101
Flight Enclosure Identity	1.51 [0.34, 4.19]	5.06 [0.96, 14.19]	3658/3486
Bird Identity: Time Post Stress Exposure (Control)	0.36 [0.11, 0.56]	0.49 [0.14, 0.90]	3397/3496
Bird Identity: Time Post Stress Exposure (Stress Exposed)	0.46 [0.21, 0.81]	0.71 [0.28, 1.26]	3459/3390
Residual Variance and Repeatability			
σ_{Control}	1.21 [1.19, 1.24]	2.09 [2.06, 2.13]	3420/3917
$\sigma_{\text{Stress exposure}}$	1.18 [1.14, 1.22]	2.06 [1.99, 2.12]	3542/3679
R _{Control}	0.07 [0.01, 0.18]	0.06 [0.01, 0.16]	3420/3917
R _{Stress exposure}	0.14 [0.03, 0.32]	0.11 [0.02, 0.27]	3542/3679

TABLE 2 Chronic effects of stress exposure on eye region temperature (T_s) and dry heat transfer (q_{Tot}) of black-capped chickadees across ambient temperature ($n = 19$; $n = 9$ females, $n = 10$ males); results of a hierarchical, Bayesian GAMMs. Results for each level of tensor products are separated by semicolons. Obelisks (\dagger) represent smooth terms, for which estimates refer to the degree of smoothness (ϕ : 0 = linear slope). Estimates for remaining population-level terms represent linear slopes, whilst those for group-level effects represent standard deviation explained by respective terms. Again, degree of smoothness and 95% credible intervals ("CIs") for tensor products represent means across penalisation groupings, and effective sample sizes represent sums across groupings. Eye region temperature measurements were estimated from infrared thermographic images ($n = 5832$) captured across 60 days. T_s model: $R^2 = 0.85$; q_{Tot} model: $R^2 = 0.94$. Asterisks (*) represent statistically significant terms (95% credible intervals do not cross zero).

Population-level Predictors			
Term	T _s Estimate [95% CIs]	q _{Tot} [95% CIs]	Effective Sample Size (T _s /q _{Tot})
Intercept*	32.90 [30.73, 34.75]	18.68 [14.87, 25.23]	3479/3419
Treatment	0.02 [-0.16, 0.20]	0.00 [-0.29, 0.29]	3628/3370
Sex (Male)	0.02 [-0.41, 0.44]	0.03 [-0.71, 0.76]	3387/3628
†Ambient Temperature*	1.57 [0.31, 5.31]	1.28 [0.12, 4.76]	3742/3425
†Ambient Temperature: Treatment*	1.81 [0.32, 5.58]	2.51 [0.61, 6.92]	3608/3299
†Hour \otimes Orientation*	3.41 [0.72, 8.48]	4.17 [0.81, 9.51]	7206/6862
Group-level Predictors			
Bird Identity	0.35 [0.23, 0.57]	0.62 [0.40, 1.00]	3551/3263
Date of Photo	1.83 [1.40, 2.36]	3.31 [2.56, 4.26]	3598/3470
Flight Enclosure Identity	1.50 [0.31, 4.36]	4.85 [0.81, 13.86]	3467/3508
Bird Identity: Ambient Temperature (Control)	0.88 [0.54, 1.36]	1.76 [1.09, 2.74]	3633/3507
Bird Identity: Ambient Temperature (Stress exposure)	1.52 [0.85, 2.42]	3.05 [1.82, 4.74]	3608/3458
Residual Variance and Repeatability			
σ_{Control}	1.20 [1.18, 1.23]	2.07 [2.04, 2.11]	3503/3846
$\sigma_{\text{Stress exposure}}$	1.17 [1.13, 1.21]	2.04 [1.98, 2.10]	3297/3461
R _{Control}	0.34 [0.17, 0.56]	0.41 [0.22, 0.63]	3503/3846
R _{Stress exposure}	0.61 [0.35, 0.81]	0.67 [0.44, 0.84]	3297/3461

Figures



FIGURE 1 Depiction of experimental stress exposure (novel object) and infrared thermographic imaging in a selected flight enclosure. Black-capped chickadees ($n = 5$) within a given flight enclosure were simultaneously exposed to each individual stressor (here, the presence of a garden gnome), whilst individuals at raised feeding platforms were passively imaged with a remotely activated infrared thermographic camera.

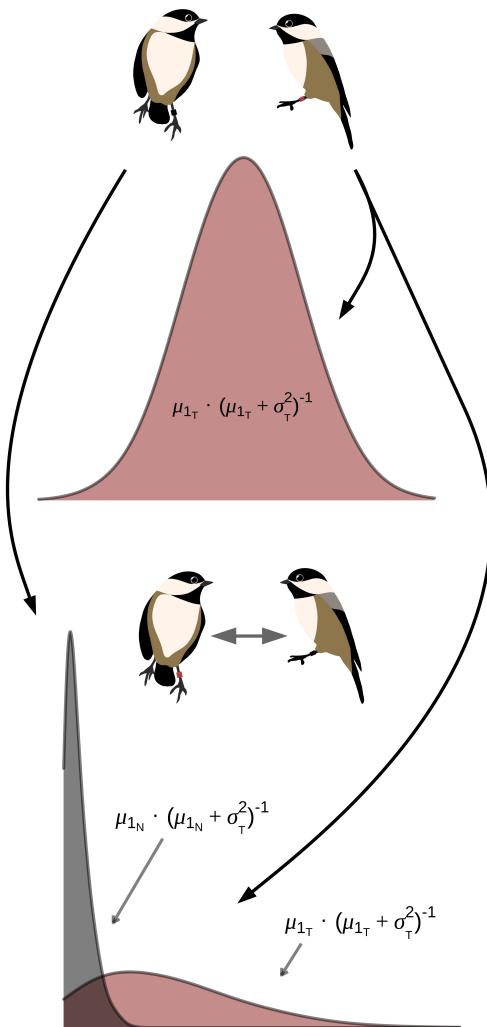


FIGURE 2 Method used to test for repeatability of stress-induced thermal responses among black-capped chickadees, whilst controlling for possible biases in the experimental process. Repeatability values were calculated from a true model (maroon; subscripted "T") using methods described by Araya-Ajoy et al (2015). Individual identities were then scrambled to produce a null model (grey; subscripted "N"), from which repeatability values were again calculated as described above. Final repeatability estimates from true and null models were compared statistically.

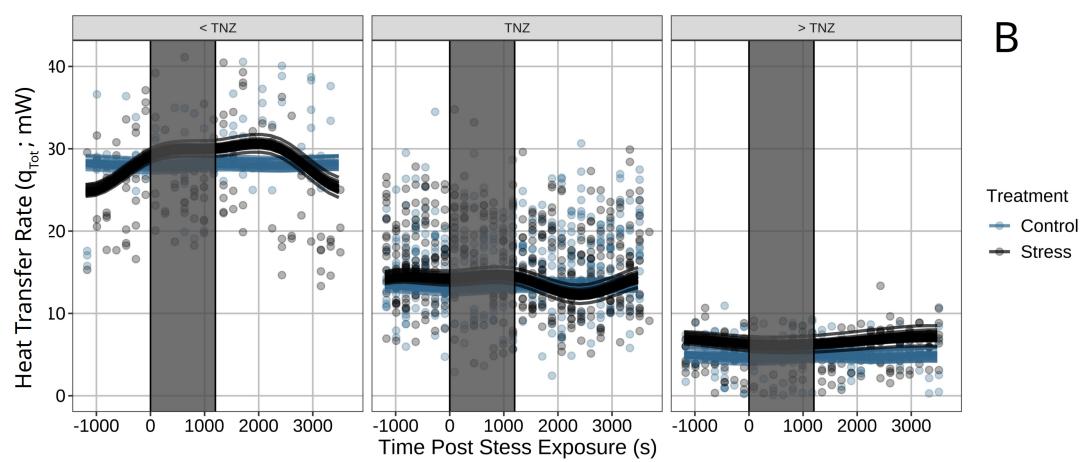
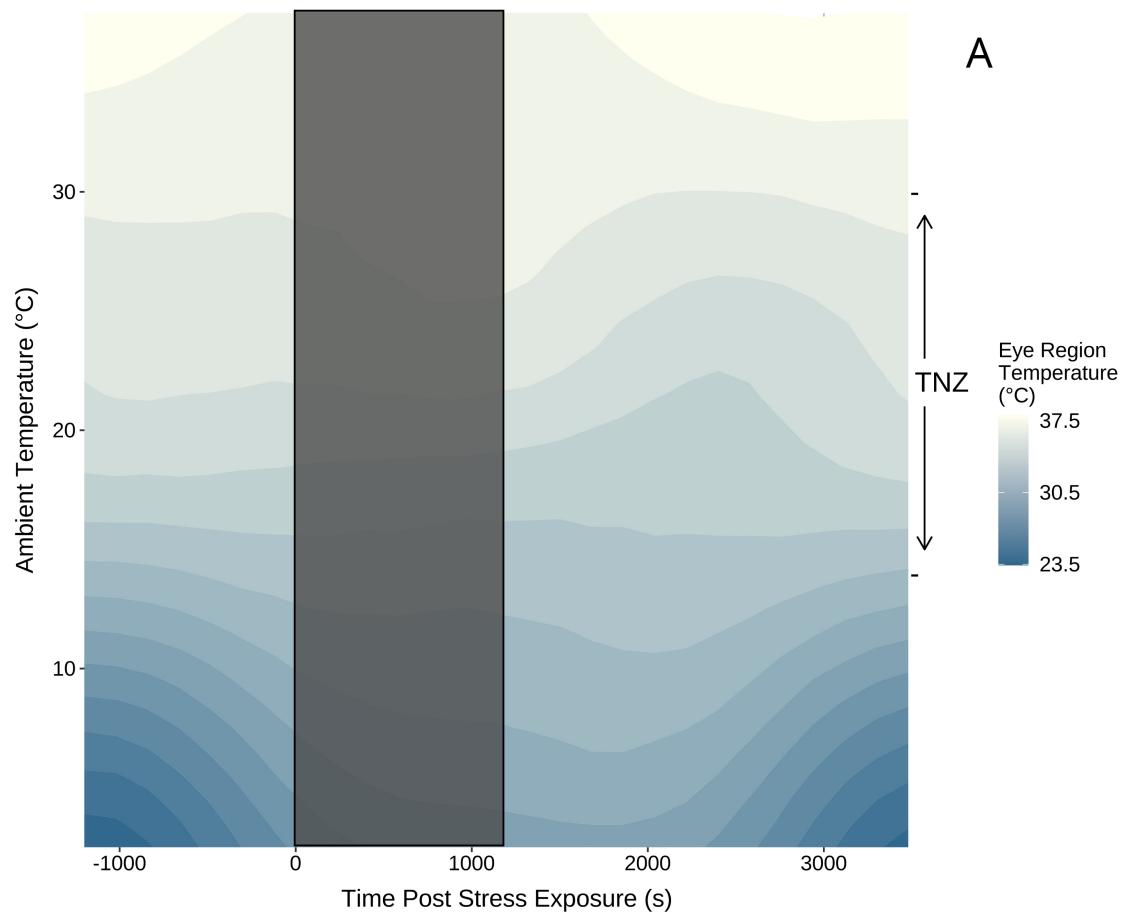


FIGURE 3 Acute changes in eye region temperature (T_s) and dry heat transfer (q_{Tot}) following stress exposure in black-capped chickadee ($n = 19$) across ambient temperature. A | Average change in T_s following stress exposure across ambient temperature ($^{\circ}\text{C}$) and time since exposure (s). Averages are derived from a Bayesian generalised additive mixed effects model (GAMM) and are marginalised across all other model predictors. T_s decreases after stress exposure at ambient temperatures below thermoneutrality, and increases after stress exposure at ambient temperatures above thermoneutrality. B | Changes in q_{Tot} of black-capped chickadees across both control and stress-exposed treatments, where slopes per treatment are permitted to vary among individuals. Each line represents the trend for a given individual at temperatures below, within, and above the thermoneutral zone (TNZ; estimated from Grossman and West, 1977), as predicted from a Bayesian GAMM. Dots represent averages per individual across 3 minutes of observation. Both trend lines and dots represent averages for each ambient temperature grouping (< TNZ, TNZ, > TNZ). Grey rectangles in panels A and B represent time when stress exposure treatments were applied in stress-exposed treatment groups. T_s and q_{Tot} were estimated by infra-red thermography ($n = 5832$ images) across 60 days.

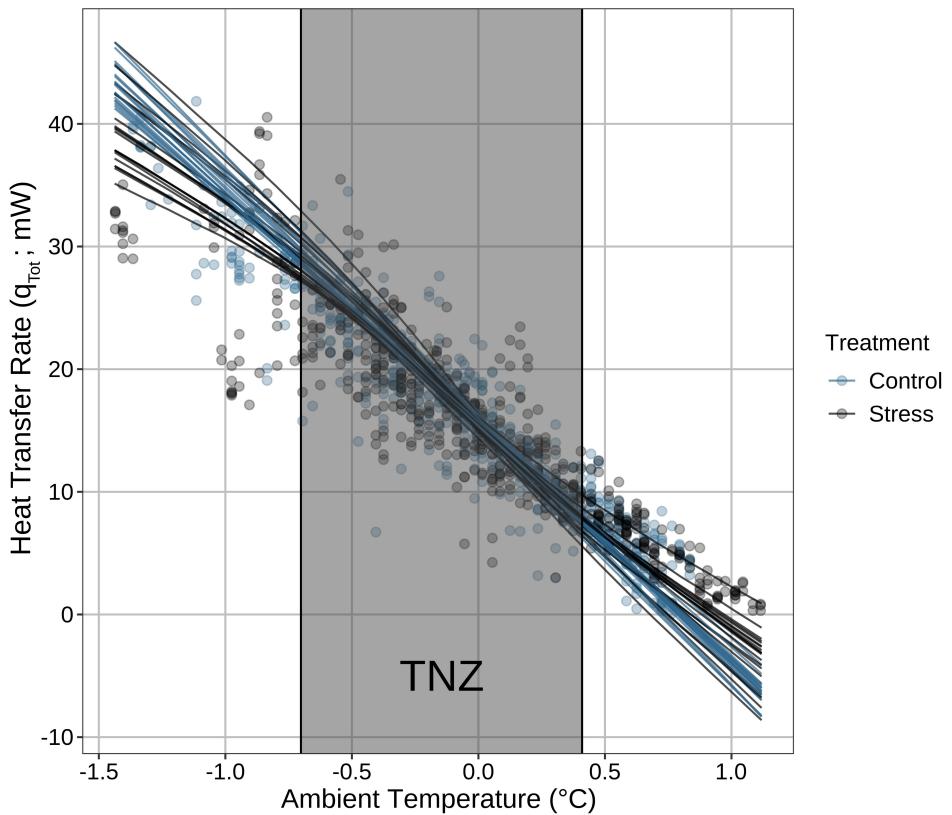


FIGURE 4 Chronic changes in dry heat transfer (q_{Tot}) at the eye region of black-capped chickadees ($n = 19$) following stress exposure across varying ambient temperatures. Individual lines represents the predicted correlation between ambient temperature (here, mean-centered) and q_{Tot} of individual black-capped chickadees during stress-exposure or control treatments. Grey rectangle represents the thermoneutral zone (TNZ) for black-capped chickadees (estimated from Grossman and West, 1977). Correlations are estimated from a Bayesian generalised additive mixed effects model (GAMM) and marginalised across all environmental and experimental parameters. q_{Tot} values were estimated by infra-red thermography ($n = 5832$ images) across 60 days.

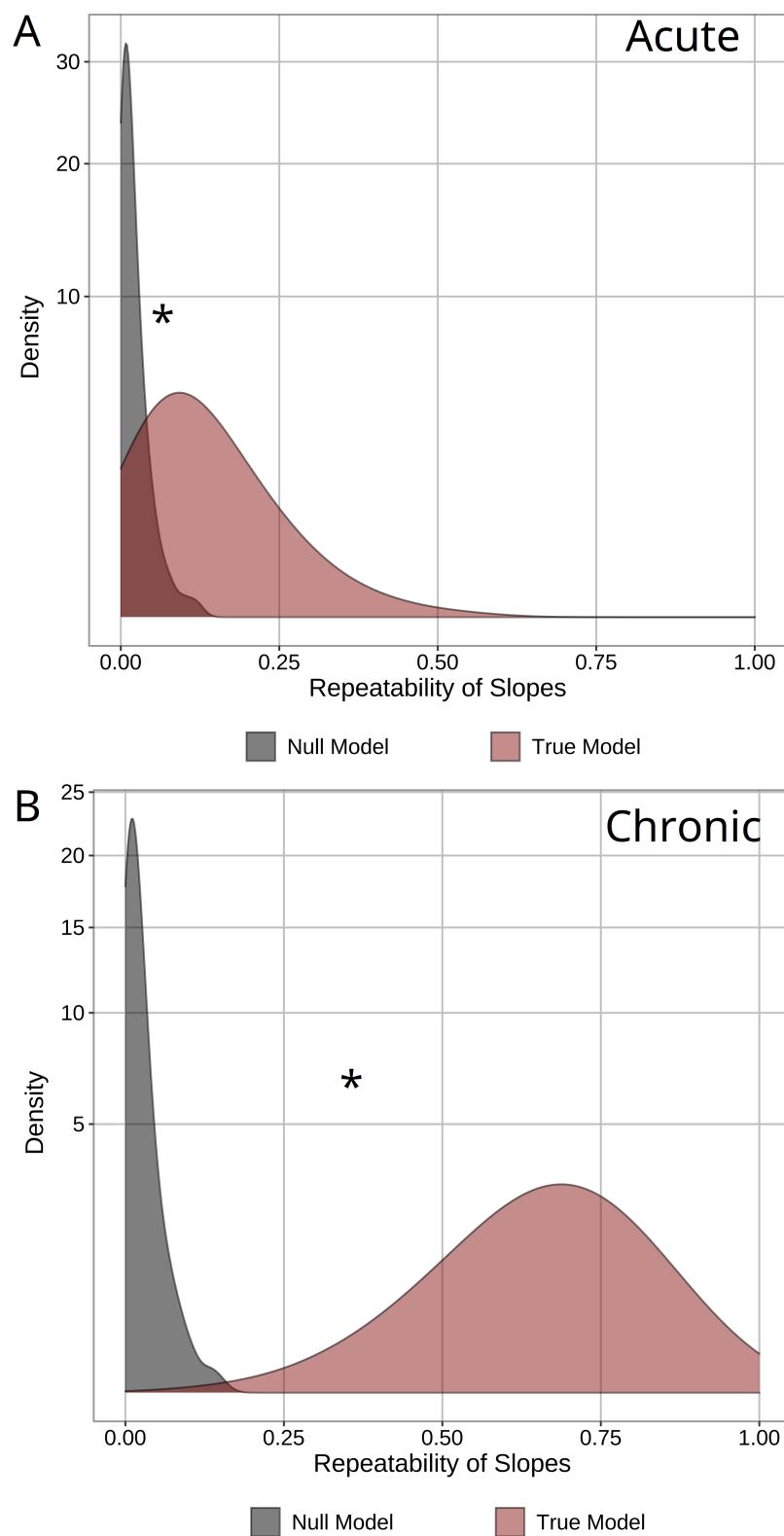


FIGURE 5 Repeatability of acute and chronic changes in dry heat transfer (q_{Tot}) at the eye region during stress exposure in black-capped chickadees ($n = 19$). Panels A and B represent distribution of repeatability values for acute and chronic responses to stress exposure, respectively. True model distributions (red) represent those drawn from models where identity of individuals was correctly identified. In contrast, null model distributions (grey) represent those drawn from models where identity of individuals was randomly scrambled. A positive difference between true and null distributions (indicated by an asterisk, "") implies that repeatability values from true models cannot be explained by biases in experimental methods (captured in null models) and are considered significant. Distributions are estimated from posteriors of Bayesian generalised additive mixed effects models (GAMM). Thermal responses to stress exposure represent those observed at the eye region of chickadees, using infra-red thermography across 60 days of observation.

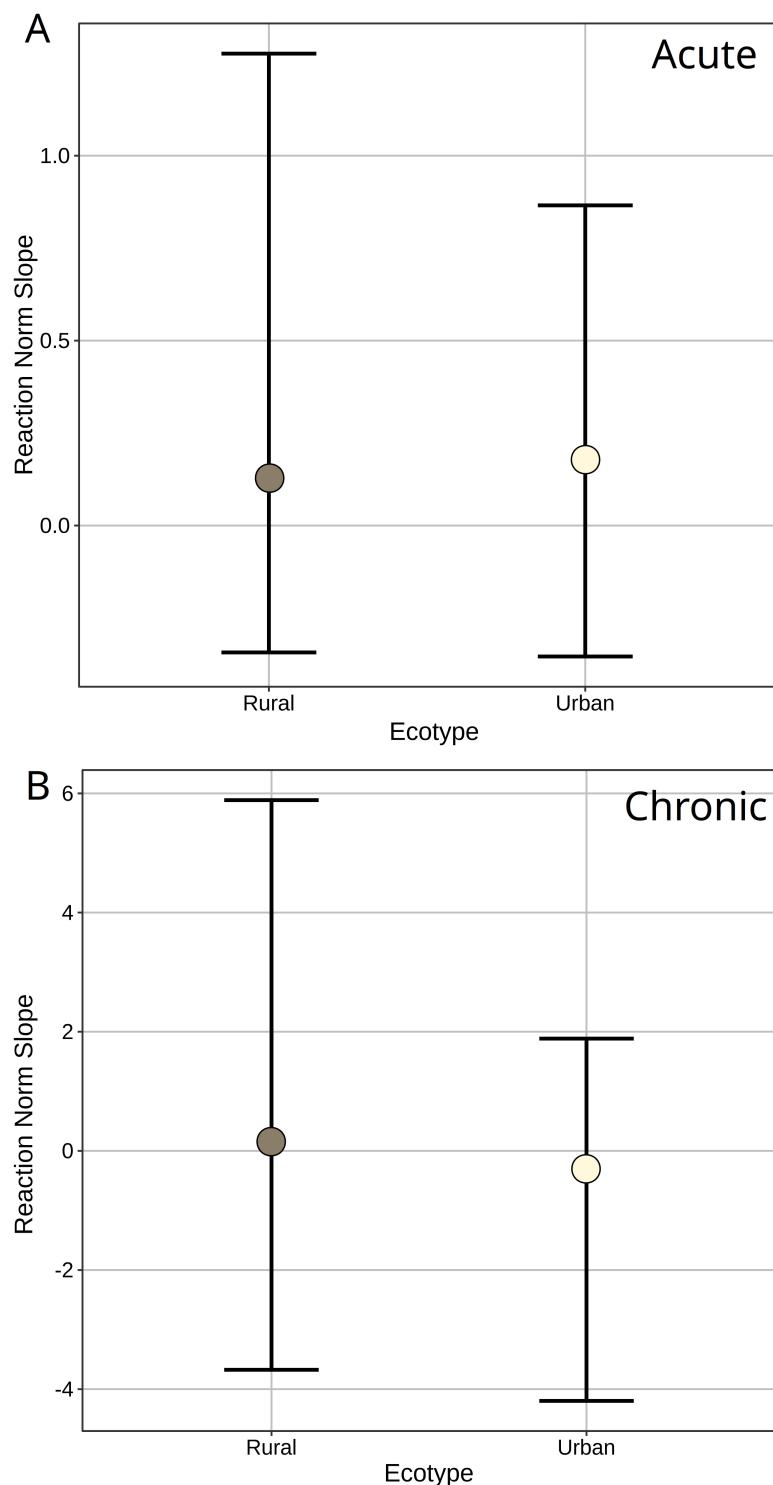


FIGURE 6 Average effect of stress exposure on dry heat transfer (q_{Tot} ; reaction norm slopes) at the eye region of black-capped chickadees ($n = 19$) captured from urban and rural ecotypes ($n = 9$ urban, $n = 10$ rural). A | Average slopes of acute reaction norm across individuals captured at each ecotype. Reaction norm slopes represent the slopes of the linear interaction between treatment type and time post stress exposure (s) per individual. B | Average slopes of chronic reaction norms across individuals captured from each ecotype. Here, reaction norm slopes represent those of linear interactions between treatment type and ambient temperature ($^{\circ}\text{C}$) per individual. Error bars represent 95% credible intervals around mean estimates. All reaction norm slopes were derived from Bayesian generalised additive mixed effects models (GAMMs).

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